

College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2017 Nov 7. pii: S0735-1097(17)41517-8. doi: 10.1016/j.jacc.2017.11.004; and Whelton *et al.* (2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2017 Nov 7. pii: S0735-1097(17)41519-1. doi: 10.1016/j.jacc.2017.11.006).

Despite the number of anti-hypertensive drugs available for treating hypertension, more than two-thirds of subjects are not controlled with one anti-hypertensive agent and require two or more anti-hypertensive agents selected from different drug classes. This further reduces the number of subjects with controlled blood pressure as adherence is reduced and side-effects are increased with increasing numbers of medications.

SUMMARY OF THE INVENTION

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding angiotensinogen (AGT). The AGT gene may be within a cell, *e.g.*, a cell within a subject, such as a human subject. The present invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of an AGT gene and/or for treating a subject who would benefit from inhibiting or reducing the expression of an AGT gene, *e.g.*, a subject suffering or prone to suffering from an AGT-associated disorder, *e.g.*, hypertension.

Accordingly, in an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of angiotensinogen (AGT) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3 and the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the corresponding portion of the nucleotide sequence of SEQ ID NO:2 or SEQ ID NO:4.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of angiotensinogen (AGT) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides from the nucleotide sequence 5'- asgsaagcUfgCfCfGfuuguucuggu -3' of SEQ ID NO:624, and the antisense strand comprises at least 15 contiguous nucleotides differing from the nucleotide sequence 5'- asCfscagAfaCfAfacggCfaGfcuucususc -3' of SEQ ID NO:926,

wherein a, g, c, and u are 2'-O-methyl (2'-OMe) A, G, C, and U, respectively; Cf is 2'-fluorocytidine-3'-phosphate; Uf is 2'-fluorouridine-3'-phosphate; Gf is 2'-fluoro guanosine-3'-

phosphate; Af is 2'-fluoroadenosine-3'-phosphate; and s is a phosphorothioate linkage.

In another aspect, the present invention provides a cell containing the dsRNA agent, or a salt thereof, as described herein.

5 In another aspect, the present invention provides a pharmaceutical composition for inhibiting expression of a gene encoding angiotensinogen (AGT) comprising the dsRNA agent, or a salt thereof, as described herein and a pharmaceutically acceptable carrier.

10 In another aspect, the present invention provides a use of the dsRNA agent, or a salt thereof, as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating a subject having a disorder that would benefit from reduction in angiotensinogen (AGT) expression, wherein the medicament is to be administered to the subject in a therapeutically effective amount.

15 In another aspect, the present invention provides a use of the dsRNA agent, or a salt thereof, as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for preventing at least one symptom in a subject having a disorder that would benefit from reduction in angiotensinogen (AGT) expression, wherein the medicament is to be administered to the subject in a prophylactically effective amount.

In another aspect, the present invention provides a kit comprising the dsRNA agent, or a salt thereof, as described herein or the pharmaceutical composition as described herein.

20 In another aspect, the present invention provides a vial comprising the dsRNA agent, or a salt thereof, as described herein or the pharmaceutical composition as described herein.

In another aspect, the present invention provides a syringe comprising the dsRNA agent, or a salt thereof, as described herein or the pharmaceutical composition as described herein.

In another aspect, the present invention provides an RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agent, or a salt thereof, as described herein.

25 In another aspect, the present invention provides an *in vitro* method of inhibiting expression of an angiotensinogen (AGT) gene in a cell, the method comprising contacting the cell with the dsRNA agent, or a salt thereof, as described herein, or the pharmaceutical composition as described herein, thereby inhibiting expression of the AGT gene in the cell.

30 In another aspect, the present invention provides the dsRNA agent, or a salt thereof, as described herein, or the pharmaceutical composition as described herein for use in therapy.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of angiotensinogen (AGT) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding AGT, and wherein the region of complementarity comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing
35 by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-7.

We claim:

- 5 1. A double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of angiotensinogen (AGT) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,
- wherein the sense strand comprises at least 15 contiguous nucleotides from the nucleotide sequence 5'- asgsaagcUfgCfCfGfuuguucuggu -3' of SEQ ID NO:624, and the antisense strand
- 10 comprises at least 15 contiguous nucleotides differing from the nucleotide sequence 5'- asCfscagAfaCfAfacggCfaGfcuucususc -3' of SEQ ID NO:926,
- wherein a, g, c, and u are 2'-O-methyl (2'-OMe) A, G, C, and U, respectively; Cf is 2'-fluorocytidine-3'-phosphate; Uf is 2'-fluorouridine-3'-phosphate; Gf is 2'-fluoro guanosine-3'-phosphate; Af is 2'-fluoroadenosine-3'-phosphate; and s is a phosphorothioate linkage.
- 15 2. The dsRNA agent, or a salt thereof, of claim 1, wherein the sense strand comprises at least 17 contiguous nucleotides from the nucleotide sequence 5'- asgsaagcUfgCfCfGfuuguucuggu -3' of SEQ ID NO:624, and the antisense strand comprises at least 17 contiguous nucleotides differing from the nucleotide sequence 5'- asCfscagAfaCfAfacggCfaGfcuucususc -3' of SEQ ID NO:926.
- 20 3. The dsRNA agent, or a salt thereof, of claim 1, wherein the sense strand comprises the nucleotide sequence 5'- asgsaagcUfgCfCfGfuuguucuggu -3' of SEQ ID NO:624, and the antisense strand comprises the nucleotide sequence 5'- asCfscagAfaCfAfacggCfaGfcuucususc -3' of SEQ ID NO:926.
- 25 4. The dsRNA agent, or a salt thereof, of claim 1, wherein the sense strand consists of the nucleotide sequence 5'- asgsaagcUfgCfCfGfuuguucuggu -3' of SEQ ID NO:624, and the antisense strand consists of the nucleotide sequence 5'- asCfscagAfaCfAfacggCfaGfcuucususc -3' of SEQ ID NO:926.
- 30 5. The dsRNA agent, or a salt thereof, of claim 3, wherein the double stranded region is 21-23 nucleotide pairs in length.
6. The dsRNA agent, or a salt thereof, of any one of claims 1-5, wherein each strand is
- 35 independently no more than 30 nucleotides in length.

7. The dsRNA agent, or a salt thereof, of any one of claims 1-6, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

8. The dsRNA agent, or a salt thereof, of any one of claims 1-6, wherein at least one strand
5 comprises a 3' overhang of at least 2 nucleotides.

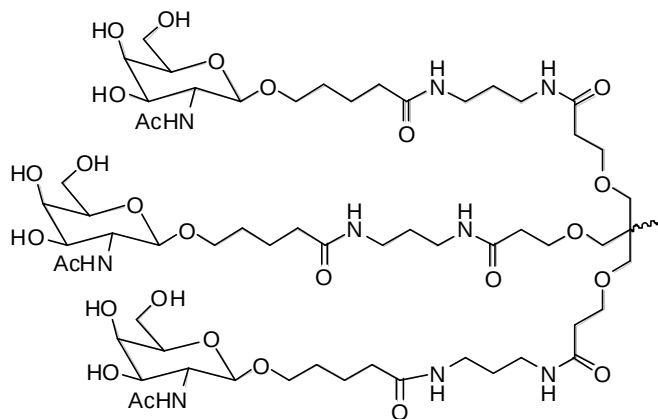
9. The dsRNA agent, or a salt thereof, of any one of claims 1-8, further comprising a ligand.

10. The dsRNA agent, or a salt thereof, of claim 9, wherein the ligand is conjugated to the 3'
10 end of the sense strand of the dsRNA agent.

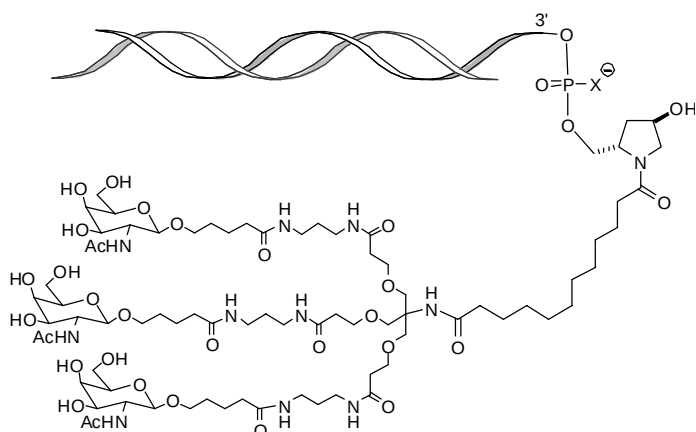
11. The dsRNA agent, or a salt thereof, of claim 9 or 10, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

12. The dsRNA agent, or a salt thereof, of any one of claims 9-11, wherein the ligand is one or
15 more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

13. The dsRNA agent, or a salt thereof, of claim 11 or 12, wherein the ligand is



14. The dsRNA agent, or a salt thereof, of claim 13, wherein the dsRNA agent is conjugated to
20 the ligand as shown in the following schematic



and, wherein X is O or S.

15. The dsRNA agent, or a salt thereof, of claim 14, wherein X is O.

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16. A cell containing the dsRNA agent, or a salt thereof, of any one of claims 1-15.

17. A pharmaceutical composition for inhibiting expression of a gene encoding angiotensinogen (AGT) comprising the dsRNA agent, or a salt thereof, of any one of claims 1-15 and a pharmaceutically acceptable carrier.

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18. The pharmaceutical composition of claim 17, wherein dsRNA agent, or a salt thereof, is in an unbuffered solution.

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19. The pharmaceutical composition of claim 18, wherein the unbuffered solution is saline or water.

20. The pharmaceutical composition of claim 17, wherein said dsRNA agent, or a salt thereof, is in a buffer solution.

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21. The pharmaceutical composition of claim 20, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

22. The pharmaceutical composition of claim 21, wherein the buffer solution is phosphate buffered saline (PBS).

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23. Use of the dsRNA agent, or a salt thereof, of any one of claims 1-15, or the pharmaceutical composition of any one of claims 17-22 in the manufacture of a medicament for treating a subject having a disorder that would benefit from reduction in angiotensinogen (AGT) expression, wherein the medicament is to be administered to the subject in a therapeutically effective amount.

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24. Use of the dsRNA agent, or a salt thereof, of any one of claims 1-15, or the pharmaceutical composition of any one of claims 17-22 in the manufacture of a medicament for preventing at least one symptom in a subject having a disorder that would benefit from reduction in angiotensinogen (AGT) expression, wherein the medicament is to be administered to the subject in a prophylactically effective amount.

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25. The use of claim 23 or 24, wherein the disorder is an AGT-associated disorder.

26. The use of claim 25, wherein the AGT-associated disorder is selected from the group consisting of high blood pressure, hypertension, borderline hypertension, primary hypertension, secondary hypertension isolated systolic or diastolic hypertension, pregnancy-associated hypertension, diabetic hypertension, resistant hypertension, refractory hypertension, paroxysmal hypertension, renovascular hypertension, Goldblatt hypertension, hypertension associated with low plasma renin activity or plasma renin concentration, ocular hypertension, glaucoma, pulmonary hypertension, portal hypertension, systemic venous hypertension, systolic hypertension, labile hypertension; hypertensive heart disease, hypertensive nephropathy, atherosclerosis, arteriosclerosis, vasculopathy, diabetic nephropathy, diabetic retinopathy, chronic heart failure, cardiomyopathy, diabetic cardiac myopathy, glomerulosclerosis, coarctation of the aorta, aortic aneurism, ventricular fibrosis, heart failure, myocardial infarction, angina, stroke, renal disease, renal failure, systemic sclerosis, intrauterine growth restriction (IUGR) , fetal growth restriction, obesity, liver steatosis/ fatty liver, non-alcoholic Steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD); glucose intolerance, type 2 diabetes (non-insulin dependent diabetes), and metabolic syndrome.

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27. The use of claim 26, wherein the subject has a systolic blood pressure of at least 130 mm Hg or a diastolic blood pressure of at least 80 mm Hg.

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28. The use of claim 26, wherein the subject has a systolic blood pressure of at least 140 mm Hg and a diastolic blood pressure of at least 80 mm Hg.

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29. The use of claim 26, the subject is part of a group susceptible to salt sensitivity, is overweight, is obese, or is pregnant.
30. The use of any one of claims 23-29, wherein the subject is a human.
- 5 31. The use of any one of claims 23-30, wherein administration of the dsRNA agent, or a salt thereof, to the subject causes a decrease in AGT protein accumulation in the subject.
- 10 32. The use of any one of claims 23-31, wherein the dsRNA agent, or a salt thereof, is to be administered to the subject subcutaneously.
33. The use of any one of claims 23-32, further comprising determining the level of AGT in a sample from the subject.
- 15 34. The use of claim 33, wherein the level of AGT in the subject sample is an AGT protein level in a blood or serum or urine or liver tissue sample.
35. The use of any one of claims 23-34, wherein the level of bradykinin, prekallikrein, or blood pressure is to be further determined in the subject.
- 20 36. The use of any one of claims 23-34, wherein an additional therapeutic agent for treatment of an AGT-associated disorder is to be further administered to the subject.
- 25 37. The use of claim 36, wherein the additional therapeutic agent is selected from the group consisting of a diuretic, an angiotensin converting enzyme (ACE) inhibitor, an angiotensin II receptor antagonist, a beta-blocker, a vasodilator, a calcium channel blocker, an aldosterone antagonist, an alpha2-agonist, a renin inhibitor, an alpha-blocker, a peripheral acting adrenergic agent, a selective D1 receptor partial agonist, a nonselective alpha-adrenergic antagonist, a steroidal antimineralocorticoid agent, an angiotensin receptor-neprilysin inhibitor (ARNi),
30 sacubitril/valsartan; or an endothelin receptor antagonist (ERA), sitaxentan, ambrisentan, atrasentan, BQ-123, zibotentan, bosentan, macitentan, and tezosentan; a combination of any of the foregoing; and a hypertension therapeutic agent formulated as a combination of agents.
- 35 38. The use of claim 36, wherein the additional therapeutic agent comprises an angiotensin II receptor antagonist.

39. The use of claim 38, wherein the angiotensin II receptor antagonist is selected from the group consisting of losartan, valsartan, olmesartan, eprosartan, and azilsartan.

5 40. A kit comprising the dsRNA agent, or a salt thereof, of any one of claims 1-15 or the pharmaceutical composition of any one of claims 17-22.

41. A vial comprising the dsRNA agent, or a salt thereof, of any one of claims 1-15 or the pharmaceutical composition of any one of claims 17-22.

10 42. A syringe comprising the dsRNA agent, or a salt thereof, of any one of claims 1-15 or the pharmaceutical composition of any one of claims 17-22.

15 43. An RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agent, or a salt thereof, of any one of claims 1-15.

44. An *in vitro* method of inhibiting expression of an angiotensinogen (AGT) gene in a cell, the method comprising contacting the cell with the dsRNA agent, or a salt thereof, of any one of claims 1-15, or the pharmaceutical composition of any one of claims 17-22, thereby inhibiting expression of the AGT gene in the cell.

20 45. The dsRNA agent of the dsRNA agent, or a salt thereof, of any one of claims 1-15, or the pharmaceutical composition of any one of claims 17-22 for use in therapy.